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REVIEW

Microalgae and wastewater treatment

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Abstract Organic and inorganic substances which were released into the environment as a result of domestic, agricultural and industrial water activities lead to organic and inorganic pollution. The normal primary and secondary treatment processes of these wastewaters have been introduced in a growing number of places, in order to eliminate the easily settled materials and to oxidize the organic material present in wastewater. The final result is a clear, apparently clean effluent which is discharged into natural water bodies. This secondary effluent is, however, loaded with inorganic nitrogen and phosphorus and causes eutrophication and more long-term problems because of refractory organics and heavy metals that are discharged. Microalgae culture offers an interesting step for wastewater treatments, because they provide a tertiary biotreatment coupled with the production of potentially valuable biomass, which can be used for several purposes. Microalgae cultures offer an elegant solution to tertiary and quandary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth. And also, for their capacity to remove heavy metals, as well as some toxic organic compounds, therefore, it does not lead to secondary pollution. In the current review we will highlight on the role of micro-algae in the treatment of wastewater.

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Contents

1. Introduction

It is truism nowadays to recognize that pollution associated problems are a major concern of society. Environmental laws are given general applicability and their enforcement has been increasingly stricter. So, in terms of health, environment and economy, the fight against pollution has become a major issue.

Today, although the strategic importance of fresh water is universally recognized more than ever before, and although issues concerning sustainable water management can be found almost in every scientific, social, or political agenda all over the world, water resources seem to face severe quantitative and qualitative threats. The pollution increase, industrialization and rapid economic development, impose severe risks to availability and quality of water resources, in many areas worldwide.

The problems of water shortage in the Middle East and North Africa (MENA) regions are well documented. Most countries in this region are arid or semi-arid. They have low rainfall, mostly with seasonal and erratic distribution. The MENA region, home up to 5% of the world's people contains less than 1% of the world's annual renewable freshwater. On the other hand, water demand in arid and semi-arid countries is growing fast. The population, having more than doubled in the past 30 years to about 280 million, could double again in the next 30 years. Cities' growing at more than 4% a year already contain 60% of the region's people. As population has grown against a background of finite freshwater resources, so the water available to individuals has fallen dramatically. Annual per capita availability, about 3300 cubic meters in 1960, has fallen by 60% to about 1250 cubic meters in 1995, the lowest in the world, and it is predicted to fall by another 50% to about 650 cubic meters by 2025.

For the region as a whole, agriculture is the largest user of water (87%), while industry and domestic supplies consume 7% and 8%, respectively (El-Gohary, 2001; Samhan, 2008).

Water quality degradation is quickly joining water scarcity as a major issue in the region. The relative severity of water quality varies among Middle East countries according to a number of factors, including population growth and density, extent of industrialization quality of non-renewable water resources, economic situation and institutional capacity.

Pollution is a man-made phenomenon, arising either when the concentrations of naturally occurring substances are increased or when non-natural synthetic compounds (xenobiotics) are released into the environment. Organic and inorganic substances which are released into the environment as a result of domestic, agricultural and industrial water activities lead to organic and inorganic pollution (Mouchet, 1986; Lim et al., 2010).

There are still a number of cases whereby municipal and rural domestic wastewater is discharged directly into water-ways, often without treatment. The discharges are increasing year after year due to the existing plan for water supply networks set-up in many villages. Also, the present expansion of water networks in several towns without parallel construction of new sewage systems or rehabilitation of the existing ones aggravated the problems and lead to pollution problems of the water bodies and increasing public health hazards. The constituents of domestic and urban input to water resources are pathogens, nutrients, suspended solids, salts and oxygen demanding materials.

One of the major sources of water pollution is the uncontrolled discharge of human wastes, while some countries have made massive investment in water supply projects there has been an overall under-investment in appropriate sanitation systems, which has resulted in harmful contamination of water resources, increased flooding and reduced health benefits from water investments. Finding a solution for the treatment and safe discharge of the wastewater is a difficult challenge because it entails integrated processes in which technical, economic and financial consideration come in play. The uniqueness of each situation makes it difficult to define a universal method for selecting the most adequate type of waste treatment plant. However, it is important to ensure that appropriate treatment standards are selected to meet local conditions, and alternative innovative technologies for treating wastewater are considered. Both conventional and innovative methods should be evaluated.

Overall the agricultural drains receive the bulk of the treated and untreated domestic pollution load. As a result many canals now also are contaminated with wastewater pollutants. A part from being the largest consumer of water, agriculture is also a major water polluter. Saline irrigation return-flows or drainage containing agrochemical residues are serious contaminants for downstream water users. Agricultural nitrate is contaminating groundwater. The disposal of liquid animal waste pollutes surface and groundwater, etc. This means a large number of organic and inorganic substances disturb the water quality, which are the main causes of eutrophication of the water body. They also proved to be powerful stimulants to algal growth and consequently formation of "algal blooms". Algal blooms can affect the water quality in several ways.

2. Composition of typical wastewater

Watercourses receive pollution from many different sources, which vary both in strength and volume. The composition of wastewater is a reflection of the life styles and technologies practiced in the producing society (Gray, 1989). It is a complex mixture of natural organic and inorganic materials as well as man-made compounds. Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids, and volatile acids. The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Tebbutt, 1983; Horan, 1990; Lim et al., 2010).

Different sources of pollutants include "Discharge of either raw or treated sewage from towns and villages; discharge from manufacturing or industrial plants; run-off from agricultural land; and leachates from solid waste disposal sites" these sites of pollution have problems so that a solution is sought (Horan, 1990). Scarcity of water, the need for energy and food are forcing us to explore the feasibility of wastewater recycling and resource recovery (De la Noüe and De Pauw, 1988).

3. Microbiological composition of sewage

Wastewater environment is an ideal media for a wide range of microorganisms specially bacteria, viruses and protozoa. The majority is harmless and can be used in biological sewage treatment, but sewage also contains pathogenic microorganisms,

Figure 1 Wastewater treatment station model.

which are excreted in large numbers by sick individuals and a symptomic carrier. Bacteria which cause cholera, typhoid and tuberculosis; viruses which cause infectious hepatitis; protozoa which cause dysentery and the eggs of parasitic worms are all found in sewage (Glynn Henery, 1989; Shaaban et al., 2004). The efficiency of disinfecting sewage is generally estimated by the extent of removal of total coliform organisms (Sebastian and Nair, 1984).

4. Sewage treatment processes

4.1. Conventional sewage treatment technology

In the wastewater treatment system (Fig. 1), the removal of biochemical oxygen demand (BOD), suspended solids, nutrients ($NO_3^- - N, NO_2^- - N, NH_4^+ - N$ and $PO_4^{3-} - P$), coliform bacteria, and toxicity are the main goal for getting purified wastewater. BOD exploits the ability of microorganisms to oxidize organic material to CO_2 and water using molecular oxygen as an oxidizing agent. Therefore, BOD can deplete the dissolved oxygen of receiving water leading to fish kills and anaerobiosis, hence its removal is a primary aim of wastewater treatment. Suspended solids are removed principally by physical sedimentation.

In wastewater treatment systems designed to remove nutrients, mainly dissolved nitrogen and phosphorus, is becoming an important step of treatment. Discharge of these nutrients into sensitive water bodies leads to eutrophication by stimulating the growth of unwanted plants such as algae and aquatic macrophytes. Other consequences of nitrogen compounds in wastewater effluents are toxicity of non-ionized ammonia to fish and other aquatic organisms, interference with disinfection where a free chlorine residual is required and methemoglobinemia in influents due to excessive nitrate concentrations (above 45 g/m^3) in drinking water (Lincolin and Earle, 1990). It has been concluded that single unit process is currently unavailable which can successfully and efficiently achieve all these requirements and consequently a combination is required (Horan, 1990).

4.2. Preliminary treatment of sewage

The preliminary treatment of sewage removes large solid materials delivered by sewers that could obstruct flow through the plant or damage equipment. These materials are composed of floating objects such as rags, wood, fecal material and heavier grit particles. Large floating objects can be removed by passing the sewage through bars spaced at 20–60 mm, the retained material is raked from the bars at regular intervals (Tebbutt, 1983). Grit is removed by reducing the flow velocity to a range at which grit and silt will settle, but leave organic matter in suspension, this is usually in the velocity range of 0.2–0.4 m/s (Gray, 1989).

4.3. Primary treatment of sewage

After removal of the coarse materials, sewage passes to sedimentation tanks, which aim to remove the settleable solids (represent up to 70% of the total settleable solids) by gravity. A well designed sedimentation tank can remove 40% of the BOD in the form of settleable solids (Horan, 1990). Pathogen removal during primary treatment is highly varied with various removal rates reported for different organisms (Pescod, 1986; Gray, 1989; IAWPRC study group, 1991).

4.4. Secondary treatment of sewage

The secondary treatment process aims to reduce the BOD exerted by reducing organic matter. This is mediated, primarily, by a mixed population of heterotrophic bacteria that utilize the organic constituent for energy and growth.

A large number of biological unit operations are available to achieve the aerobic oxidation of BOD. All operations can be classified on the basis of their microbial population, into either fixed film or dispersed growth processes. Fixed film reactors have biofilms attached to a fixed surface where organic compounds are adsorbed into the biofilm and aerobically degraded. In suspended (e.g. activated sludge) growth reactors the microorganisms mix freely with the wastewater and are kept in suspension by mechanical agitation or mixing by air diffusers (Horan, 1990).

Several investigators have pointed out that biological oxidation systems can remove over 90% of pathogenic bacteria from sewage, however, the removal of viruses is much more varied. The major mechanism of viral removal is thought to be adsorption. In suspended growth reactors the intimate mixing of solid flocs and sewage gives 90% removal, while the smaller surface areas of biological adsorption sites in film reactors give varied reductions (Kott et al., 1974; Lloyd and Morris, 1983; Gray, 1989; IAWPRC Study Group, 1991).

4.5. Tertiary treatment of sewage

Tertiary treatment process aims to remove all organic ions. It can be accomplished biologically or chemically. The biological tertiary treatment process appears to perform well when compared to the chemical processes which are in general too costly to be implemented in most places and which may lead to secondary pollution. In addition, each additional treatment step in a wastewater system greatly increases the total cost (Oswald, 1988b).

A complete tertiary process aimed at removing ammonium, nitrate and phosphate is estimated to be about four times more expensive than primary treatment (De la Noüe et al., 1992). Quaternary treatment intended for the removal of heavy metals, organic compounds (refactory and toxicants) and soluble minerals will be about eight to sixteen times more expensive than that of primary treatment, respectively (Oswald, 1988b).

Advanced treatments are generally based on technologically complex techniques, such as chemical precipitation, ozonation, reverse osmosis or carbon adsorption. These techniques include processes designed to remove particular nutrients, such as phosphorus or nitrogen, which can stimulate eutrophication in certain situations. For general improvements in effluent quality, especially in small-scale applications, the removal of fine particles can enable discharges below the target standards. Such systems include lagoon storage, land application and filtration through sand or gravel filters (Gray, 1989).

Some industrial and agricultural wastewater show total nitrogen and phosphorus concentrations up to three orders of magnitude higher than natural water bodies (De la Noüe et al., 1992). The normal primary and secondary treatment processes have been introduced in a growing number of places, in order to eliminate the easily settled materials (primary treatment) and to oxidize the organic material present in wastewater (secondary treatment). The final result is a clear, apparently clean effluent which is discharged into natural water bodies. This secondary effluent is, however, loaded with inorganic nitrogen and phosphorus and causes eutrophication and more long-term problems because of refractory organics and heavy metals that are discharged.

Dasilva et al. (1987), stated that, the natural purification processes identified chemoorganotrophic micro-organisms which being responsible for the destruction of the organic matter and also showed that both aerobic and anaerobic processes were operating. They also indicated that, the modern methods of the biological wastewater treatment systems still rely on the same types of self purification as those at work in the natural environment. The difference is that they are contained within installations designed to speed up the rate of treatment.

4.6. Disinfection of wastewater

Primary, secondary and even tertiary treatment cannot by expected to remove 100% of the incoming waste load and as a result, many organisms still remain in the waste stream. To prevent the spread of waterborne diseases and also to minimize public health problems, regulatory agencies may require the destruction of pathogenic organisms in wastewaters. While most of these microorganisms are not pathogens, pathogens must be assumed to be potentially present. Thus, whenever wastewater effluents are discharged into receiving waters which may be used for water supply, swimming or shellfishing, the reduction of bacterial numbers to minimize health hazards is a very desirable goal. Disinfection is the treatment of the effluent for the destruction of all pathogens. Another term that is sometimes also used in describing the destruction of microorganisms is *sterilization*. Sterilization is the destruction of *all* microorganisms. While disinfection indicates the destruction of all disease causing microorganisms, no attempt is made in wastewater treatment to obtain sterilization. However, disinfection procedures applied to wastewaters will result in a substantial reduction of all microbes so that bacterial numbers are reduced to a safe level. In general, disinfection can be achieved by any method that destroys pathogens. A variety of physical or chemical methods are capable of destroying microorganisms under certain conditions. Physical methods might include, for example, heating to boiling or incineration or irradiation with X-rays or ultraviolet rays. Chemical methods might theoretically include the use of strong acids, alcohols, or a variety of oxidizing chemicals or surface active agents (such as special detergents). However, the treatment of wastewaters for the destruction of pathogens demands the use of practical measures that can be used economically and efficiently at all times on large quantities of wastewaters which have been treated to various degrees. In the past, wastewater treatment practices have principally relied on the use of chlorine for disinfection. The prevalent use of chlorine has come about because chlorine is an excellent disinfecting chemical and, until recently, has been available at a reasonable cost. However, the rising cost of chlorine coupled with the fact that chlorine even at low concentrations is toxic to fish and other biota as well as the possibility that potentially harmful chlorinated hydrocarbons may be formed has made chlorination less favored as the disinfectant of choice in wastewater treatment. As a result, the increased use of ozone (ozonation) or ultraviolet light as a disinfectant in the future is a distinct possibility in wastewater disinfection. Both ozone and ultraviolet light, as well as being an effective disinfecting agent, leave no toxic residual. Ozone will additionally raise the dissolved oxygen level of water. However, ozone must be generated and has only recently begun to compete favorably with chlorination in terms of economics. Ultraviolet light has recently undergone studies to determine its effectiveness and cost when used at large wastewater treatment plants. While the study is not yet complete, ultraviolet light now appears effective and economically competitive with chlorination as a disinfectant. The use of both chlorine and ozone as chemical disinfectants and their disinfecting properties and actions will be considered individually. However, since chlorine continues to be used extensively as a disinfectant, we will mainly be concerned with the principles and practice of chlorination.

4.7. Aquatic systems for wastewater treatment

Serious interests in natural methods for wastewater treatment have reemerged. The using of aquaculture systems as engineered systems in wastewater (domestic and industrial) treatment and recycling has increased enormously over the past few years, they are designed to achieve specific wastewater treatment and can simultaneously solve the environmental and sanitary problems and may also be economically efficient (Bastian and Reed, 1979; O'Brien, 1981; Oron et al., 1985; Hussein et al., 2004; Deng et al., 2006).

Wastewater has been also used in a variety of aquaculture operations around the world for the production of fish or other biomass. Usually the production of biomass was a primary goal with marginal concern for wastewater renovation (Reed, 1987). The intensive growth and consequent harvesting of the algal biomass as a method for removing wastewater borne nutrients was first suggested and studied by Bogan et al. (1960). It was further investigated by Oswald and Golueke (1966) who proposed the removal of algae growth potential from wastewater by high-rate algal treatment. Large scale study in South Africa, reported by Bosman and Hendricks (1980) concerning the removal of industrial nitrogenous wastes with high-rate algal ponds concluded that a multi-stage algal system is required for exerting the full removal potential of nitrogen by algal biomass incorporation followed by algal harvesting.

Aquatic treatment systems consist of one or more shallow ponds in which one or more species of water tolerant vascular plants such as water hyacinths or duckweed are grown (Tchobanoglous, 1987). Water hyacinth systems are capable of removing high levels of BOD, suspended solids (SS), nitrogen and refractory trace organic matter (Orth and Sapkota, 1988) while phosphorus removal seldom exceeds 50–70% in wastewater, as it is mainly limited to the plant uptake (Dinges, 1976; Bastian and Reed, 1979).

A system consisting of a pond covered with duckweed mat seems to be able to purify the wastewater jointly with bacteria. The bacterial decomposition causes anaerobiosis in the water. It is maintained by the duckweed mat as it prevents reaeration. It has been shown that duckweed species such as *Spirodela* and *Lemna* even reduce the oxygen content of water (Culley and Epps, 1973) but this anaerobiosis dose not seem to affect the plants. The main minerals C, N and P in turn will be converted into protein by duckweed, also, it has the ability to remove the organic materials because of their ability to use simple organic compounds directly and assimilate them as carbohydrates and various amino acids (Hillman, 1976).

In aquatic systems used for municipal wastewater the carbonaceous biochemical oxygen demand (BOD) and the suspended solids (SS) are removed principally by bacterial metabolism and physical sedimentation. In systems used to treat BOD and SS, the aquatic plants themselves bring about very little actual treatments of wastewater (Tchobanoglous, 1987).

Many investigations have been conducted and concern the distribution and species composition of fresh water algal communities in different water supplies in Egypt in response to the impact of some environmental stresses (Abdel-Raouf et al., 2003). The polluted rivers, lakes and seas, were aesthetically displeasing also by Man which importantly were a public health hazard, since they harboured human pathogens and increased the risk of spreading excreta-related diseases through the water-borne route. In order to prevent such problems, the sewage treatment systems were designed.

Through most of human history, agriculture has been in effect a major form of biological water treatments through its use of the potential pollutants of human and animal wastes to support plant growth. Municipal sewage, for example sometimes after treatment is applied as a source of nutrients over land occupied by natural vegetation or various crops (Hunt and Lee, 1976; Wood-Well, 1977). Such wastes are still important in world agriculture, especially where commercial fertilizers are not readily available (Tourbier and Pierson, 1979).

5. Microalgae for wastewater treatment

The history of the commercial use of algal cultures spans about 75 years with application to wastewater treatment and mass production of different strains such as *Chlorella* and *Dunaliella*. Currently significant interest is developed in some advanced world nations such as Australia, USA, Thailand, Taiwan and Mexico (Borowitzka and Borowitzka, 1988, 1989a,b; Moreno et al., 1990; Wong and Chan, 1990; Renaud et al., 1994). These are due to the understanding of the biologists in these nations for the biology and ecology of large-scale algal cultures, as well as in the engineering of large-scale culture systems and algal harvesting methods, all of which are important to the design and operation of high rate algal cultures to produce high-value products, such as Pharmaceuticals and genetically engineered products (Javanmardian and Palsson, 1991). These include antibacterial, antiviral, antitumers/anticancer, antihistamine and many other biologically valuable products (Starr et al., 1962; Borowitzka, 1991; Ibraheem, 1995; Haroun et al., 1995).

Bio-treatment with microalgae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses and incorporating nutrients such as nitrogen and phosphorus causing eutrophication (De la Noüe and De Pauw, 1988). This fascinating idea launched some fifty-five years ago in the U.S. by Oswald and Gotaas (1957) has since been intensively tested in many countries (Goldman, 1979; Shelef and Soeder, 1980; De Pauw and Van Vaerenbergh, 1983).

Palmer (1974) surveyed microalgal genera from a wide distribution of waste stabilization ponds. In order of abundance, and frequency of occurrence the algae found were *Chlorella, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractinium* and *Golenkinia*.

A survey of algal taxa in six-lagoon systems in Central Asia was completed by Erganshev and Tajiev (1986). Their analysis of long term data revealed that the Chlorophyta was dominant both in variety and quantity followed by Cyanophyta, Bascillariophyta and Euglenophyta. Palmer (1969) listed the algae in the order of their tolerance to organic pollutants as reported by 165 authors. The list was compiled for 60 genera and 80 species. The most tolerant eight genera were found to be *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*. More than 1000 algal taxa have been reported one or more times as pollution tolerant which include 240 genera, 725 species and 125 varieties and forms. The most tolerant genera include eight green algae, five blue-greens, six flagellates and six diatoms.

Since the land-space requirements of microalgal wastewater treatment systems are substantial (De Pauw and Van Vaerenbergh, 1983), efforts are being made to develop wastewater treatment systems based on the use of hyperconcentrated algal cultures. This proved to be highly efficient in removing N and P within very short periods of times, e.g. less than 1 h (Lavoie and De la Noüe, 1985).

The algal systems can treat human sewage (Shelef et al., 1980; Mohamed, 1994; Ibraheem, 1998), livestock wastes (Lincoln and Hill, 1980), agro-industrial wastes (Zaid-Iso,1990; Ma et al.,1990; Phang, 1990, 1991) and industrial wastes (Kaplan et al., 1988). Also, microalgal systems for the treatment of other wastes such as piggery effluent (De Pauw et al., 1980; Martin et al., 1985a,b and Pouliot et al., 1986), the effluent from food processing factories (Rodrigues and Oliveira, 1987) and other agricultural wastes (Phang and Ong,1988) have been studied. Also, algae based system for the removal of toxic minerals such as lead, cadmium, mercury, scandium, tin, arsenic and bromine are also being developed (Soeder et al., 1978; Kaplan et al., 1988; Gerhardt et al., 1991; Hammouda et al., 1995; Cai-XiaoHua et al., 1995).

The technology and biotechnology of microalgal mass culture have been much discussed (Burlew, 1953; Barclay and Mc-Intosh, 1986; Richmond, 1986; Lembi and Waaland, 1988; Stadler et al., 1988 and Cresswell et al., 1989). Algal systems have traditionally been employed as a tertiary process (Lavoie and De la Noüe, 1985; Martin et al., 1985a; Oswald, 1988b). They have been proposed as a potential secondary treatment system (Tam and Wong, 1989).

Tertiary treatment process removes all organic ions. It can be accomplished biologically or chemically. The biological tertiary treatment appears to perform well compared to the chemical processes which are in general too costly to be implemented in most places and which may lead to secondary pollution. However, each additional treatment step in a wastewater system greatly increases the total cost. The relative cost of treatment doubles for each additional step following primary treatment (Oswald, 1988b).

A complete tertiary process aimed at removing ammonia, nitrate and phosphate will thus be about four times more expensive than primary treatment. Microalgal cultures offer an elegant solution to tertiary and quinary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth (Richmond, 1986; Oswald, 1988b,c; Garbisu et al., 1991, 1993; Tam and Wong, 1995). And also, their capacity to remove heavy metals (Rai et al., 1981), as well as some toxic organic compounds (Redalje et al., 1989), therefore, does not lead to secondary pollution. Amongst beneficial characteristics they produce oxygen, have a disinfecting effect due to increase in pH during photosynthesis (Mara and Pearson, 1986; De la Noüe and De Pauw, 1988).

Algae can be used in wastewater treatment for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removal of heavy metals.

6. Removal of coliform bacteria

Moawad (1968) observed that the environmental factors which were favourable for algal growth were unfavourable for the survival of coliforms. Pathogenic organisms of concern in wastewater include bacteria such as *Salmonella* and *Shigella*, viruses and protozoa. Bacteria provide the largest component of the microbial community in all biological wastewater treatment processes and numbers in the range of 10^6 bacteria/ml of wastewater are frequently encountered (Horan, 1990). Experimental evidence indicates that, the pathogenic bacteria generally have shorter survival times in the environment than coliforms, whereas viruses tend to survive longer.

The efficiency of disinfection of sewage is generally estimated by the extent of removal of total coliform organisms (Sebastian and Nair, 1984). In this respect sewage stabilization ponds and high-rate sewage stabilization ponds are well known for being generally more effective than conventional sewage treatment systems (Parhad and Rao, 1976; Shelef et al., 1977).

Reports in the literature revealed that, considerable coliform removal is achieved in stabilization ponds. Thus Malina and Yousef (1964) reported a reduction of 88.8% in 11.4 days. Meron et al. (1965) reported a reduction of 99.6%. Another supported study was performed in this respect (Oswald et al., 1967; Parhad and Rao, 1976).

In high-rate ponds, Shelef et al. (1977) have reported a reduction of 99% in total coliform counts. A similar observation on the percent reduction of coliforms and *Salmonella* was also made by Cooke et al. (1978), Pichai and Govindan (1980) and Colak and Kaya (1988) .

7. Reduction of both chemical and biochemical oxygen demand

As mentioned before, there are many compounds and microorganisms could be detected in wastewater, which is capable of causing the pollution of a water-course. Pollution of wastewater may be manifested in three broad categories, namely organic materials, inorganic materials in addition to microbial contents. The organic compounds of wastewater comprise a large number of compounds, which all have at least one carbon atom. These carbon atoms may be oxidized both chemically and biologically to yield carbon dioxide. If biological oxidation is employed the test is termed the Biochemical Oxygen Demand (BOD), whereas for chemical oxidation, the test is termed Chemical Oxygen Demand (COD). In other words, BOD exploits the ability of microorganisms to oxidise organic material to carbon dioxide and water using molecular oxygen as an oxidizing agent. Therefore, biochemical oxygen demand is a measure of the respiratory demand of bacteria metabolizing the organic matter present in wastewater.

Excess BOD can deplete the dissolved oxygen of receiving water leading to fish kills and anaerobiosis, hence its removal is a primary aim of wastewater treatment. Colak and Kaya (1988) investigated the possibilities of biological wastewater treatment by algae. They found that, in domestic wastewater treatment, elimination of BOD and COD were 68.4% and 67.2%, respectively.

8. Removal of N and/or P

The bio-treatment of wastewater with algae to remove nutrients such as nitrogen and phosphorus and to provide oxygen for aerobic bacteria was proposed over 50 years ago by Oswald and Gotaas (1957). Since then there have been numerous laboratory and pilot studies of this process and several sewage treatment plants using various versions of this systems have been constructed (Shelef et al., 1980; Oswald, 1988a,b; Shi et al., 2007; Zhu et al., 2008).

The nitrogen in sewage effluent arises primarily from metabolic interconversions of extraderived compounds, whereas 50% or more of phosphorus arises from synthetic detergents. The principal forms in which they occur in wastewater are NH_4^+ (ammonia), NO_2^- (nitrite), NO_3^- (nitrate) and PO_4^{3-} (orthophosphate). Together these two elements are known as nutrients and their removal is known as nutrient stripping (Horan, 1990).

Wastewater is mainly treated by aerobic or anaerobic biological degradation; however, the treated water still contains inorganic compounds such as nitrate, ammonium and phosphate ions, which leads to eutrophication in lakes and cause harmful microalgal blooms (Sawayama et al., 1998). Prased (1982) and Geddes (1984) have considered P and N to be the key of eutrophication. So, further treatment is thus necessary to prevent eutrophication of water environment (Sawayama et al., 2000).

The adverse effects of nutrient enrichment in receiving sensitive bodies of water can cause eutrophication by stimulating the growth of unwanted plants such as algae and aquatic macrophytes. Other consequences of nitrogen compounds in wastewater effluents are toxicity of non-ionized ammonia to fish and other aquatic organisms, interference with disinfection where a free chlorine residual is required and methemoglobinemia in influents due to excessive nitrate concentrations (above 45 g m^{-3}) in drinking water (Lincolin and Earle, 1990) .

Microalgal culture offers a cost-effective approach to removing nutrients from wastewater (tertiary wastewater treatment) (Evonne and Tang, 1997). Microalgae have a high capacity for inorganic nutrient uptake (Talbot and De la Noüe, 1993; Blier et al., 1995) and they can be grown in mass culture in outdoor solar bio-reactors (De la Noüe et al., 1992). Biological processes appear to perform well compared to the chemical and physical processes, which are in general, too costly to be implemented in most places and which may lead to secondary pollution (De la Noüe et al., 1992).

Microalgal cultures offer an elegant solution to tertiary and quaternary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorous for their growth (Oswald, 1988b,c; Richmond, 1986) and their capacity to remove heavy metals (Rai et al., 1981). Lau et al. (1996) studied the ability of

Chlorella vulgaris in nutrients removal and reported a nutrient removal efficiency of 86% for inorganic N and 78% for inorganic P. In earlier study, Colak and Kaya (1988) reported an elimination of nitrogen (50.2%) and phosphorus (85.7%) in industrial wastewater treatment and elimination of phosphorus (97.8%) in domestic wastewater treated by algae.

In reported papers, Lau et al. (1996) studied the ability of *Chlorella vulgaris* in the removal of nutrients. They found that the results indicated in a nutrient removal efficiency of 86% inorganic N and 70% inorganic P. In earlier study, Colak and Kaya (1988) reported an elimination of nitrogen (50.2%) and phosphorus (85.7%) in industrial wastewater treatment and elimination of phosphorus (97.8%) in domestic wastewater treated by algae.

The interest in microalgal cultures stems from the fact that conventional treatment processes suffer from some important disadvantages: (a) variable efficiency depending upon the nutrient to be removed; (b) costly to operate; (c) the chemical processes often lead to secondary pollution; and (d) loss of valuable potential nutrients (N, P) (De la Noüe et al., 1992). The last disadvantage is especially serious, because conventional treatment processes lead to incomplete utilization of natural resources (Guterstan and Todd, 1990; Phang, 1990).

Many studies demonstrated the success of using algal cultures to remove nutrients from wastewater rich in nitrogenous and phosphorus compounds (Przytocka-Jusiak et al., 1984; Rodrigues and Oliveira, 1987). Mohamed (1994) pointed out that *Scenedesmus* sp. is very common in all kinds of fresh water bodies, which play an important role as primary producers and contributes to the purification of eutrophic waters. The author indicated that the presence or absence of certain species of *Scenedesmus* can be used for the evaluation of water quality. To avoid recycling of nutrients in receiving waters, and to recover the biomass produced, harvesting or physical recovery of the algal cells is also essential, and represents one of the important technical and economic difficulties to overcome (Benemann, 1989). Indeed, most of the experiments carried out until now have used planktonic and unicellular microalgal species which are difficult to harvest (Mohn, 1980; De la Noüe and De Pauw, 1988).

Removal of inorganic compounds by using plants or microalgae has advantages of renewability and utilization of solar energy (Sawayama et al., 1998). Under suitable conditions, cyanobacteria can grow at higher rates than higher plants (Watanabe and Hall, 1996), so that inorganic nutrients-removal systems using cyanobacteria appear to have a considerable potential (Sawayama et al., 1998). With the increasing use of inorganic nitrogenous fertilizers and the production of wastes from human and animal populations, there are signs of nitrogen (N) accumulation in the environment, in the case of N pollution, most concern stems from the possible health hazards that have been attributed to nitrite either directly as a causative factor of methemoglobinemia or indirectly as the source of nitrosamines (Tam and Wong, 1989).

Also, nitrites themselves are important as precursors of N-nitroso compounds, mainly nitrosamines, which have received considerable attention due to their possible carcinogenic, teratogenic and mutagenic properties (Abel, 1989). Since nitrate is not significantly removed by conventional water treatment, much research is focused on the development of new techniques for reducing nitrates in drinking water to tolerable levels, i.e. $< 50\ \mathrm{mg\,l^{-1}}$ (World Health Organization, 1970).

Biological N removal generally appears a valid option and offers some advantages over tertiary chemical and physicochemical treatments (Proulx and De la Noüe, 1988). De la Noüe and Basseres (1989) used cultures of *Phormidium bohneri* for the removal of nitrates from the effluents obtained after anaerobic digestion of swine manure. Also, the removal of N from polluted waters using bench-top bioreactors incorporating the thermophilic cyanobacterium *Phormidium laminosum* has been reported (Garbisu et al., 1994). The usage of thermophilic cyanobacteria in wastewater purification has advantages, since contamination can be avoided because the cyanobacterium is tolerant to high temperature and can be treated at high temperatures (45 °C) (Sawayama et al., 1998).

Phormidium sp. cells were attached to chitosan flakes and used for removing N (ammonium, nitrate, nitrite) and orthophosphate from urban secondary effluents (De la Noüe and Proulx, 1988). Although to date, phosphate in water does not seem to present any problems for human health, phosphorus (P) removal from municipal and industrial wastewater is required to protect water from eutrophication (Comeau et al., 1987). Biological P removal processes have been attracting attention in the last three decades (Shaaban et al., 2004).

It is well established that in N-sufficient cells of cynobacteria the uptake and reduction of nitrate is a photosynthetically driven process which is likely to implicate product(s) of the incorporation of ammonium to carbon skeletons (Flores et al., 1980; Herrero et al., 1985; Romero et al., 1987). Also, nitrate assimilation is affected by a number of environmental and nutritional factors such as light, temperature, pH and carbon source availability, among others (Serra et al., 1990). Phosphorus and nitrogen metabolism is closely related as an abundance of phosphorus is of little use if there is no nitrogen and vice versa (Garbisu et al., 1993).

The uptake of phosphate by cyanobacteria, which has already been characterized in several strains, is an apparent hyperbolic function of the external phosphate concentration (Garbisu et al., 1993).

After the cyanobacteria have taken up the nutrients in the effluents, the purified water can be decanted and the cyanobacteria can then be harvested with ease (Talbot et al., 1990; Proulx et al., 1994). Potential end uses of the harvested biomass include the extraction of commercially valuable pigments (Mumford and Miura, 1988; Glazer, 1994).

9. Factors affecting algal growth and nutrient removal

Algal growth and nutrient uptake are not only affected by the availability of nutrients, they also depend on complex interactions among physical factors such as pH (Azov and Shelef, 1987), light intensity, temperature (Talbot and De la Noüe, 1993), and biotic factors. The first biotic factor significantly influencing algal growth is the initial density, it is expected that the higher the algal density, the better the growth and the higher the nutrient removal efficiency (Lau et al., 1995). However, the high algal density would lead to self-shading, an accumulation of autoinhibitors, and a reduction in photosynthetic efficiency (Fogg, 1975; Darley, 1982).

10. Heavy metals removal from wastewater

Microalgae are known to sequester heavy metals (Rai et al., 1981). Discharge of toxic pollutants to waste water collection systems has increased concurrently with society's progressive industrialization.

Significant concentrations of heavy metals and toxic organic compounds have been measured in municipal wastewater. Consequently, the ability of wastewater treatment systems to tolerate and remove toxicity is of considerable importance. Microalgae are efficient absorbers of heavy metals. Bioaccumulation of metals by algae may create a feasible method for remediating wastewater contaminated with metals (Nakajima et al.,1981; Darnall et al., 1986). On the other hand advantages of algae are that it may be grown in ponds with little nutritional input or maintenance.

Although the heavy metal contents in some drainage systems generally do not reach the proportions found in industrial effluents, certainly not those of metal processing industries, the problems caused by their presence, particularly in areas with dense population, are of public concern. It is well established that several marine and fresh water algae are able to take up various heavy metals selectively from aqueous media and to accumulate these metals within their cells (Afkar et al., 2010; Kumar and Gaur, 2011; Chen et al., 2012).

Several authors concluded that this method, including the separation of the metal-saturated algae from the medium, is an economic method for removing heavy metals from wastewater, resulting in high quality reusable effluent water (Filip et al., 1979; Shaaban et al., 2004; Kiran et al., 2007; Nasreen et al., 2008; Bhat et al., 2008; Pandi et al., 2009). Numerous species of algae (living and non-living cells) are capable of sequestering significant quantities of toxic heavy metal ions from aqueous solutions. Algal metal sequestering processes occur by different mechanisms. This can be dependent on the alga, the metal ion species, the solution conditions and whether the algal cells are living or nonliving. In living algal cells trace nutrient metals (such as Co, Mo, Ca, Mg, Cu, Zn, Cr, Pb and Se) are accumulated intracellularly by active biological transport (Yee et al., 2004; Han et al., 2007; Ajjabi and Chouba, 2009; Tuzen and Sari, 2010; Yuce et al., 2010;Kiran and Thanasekaran, 2011; Pipiska et al., 2011; Rajfur et al.,in press; Singh et al., 2012).

Field experiments reported by Gale (1986) indicated that, live photosynthetic microalgae have an effective role in metal detoxification of mine wastewater. By using cyanobacteria in a system of artificial pools and meanders, 99% of dissolved and particulate metals could be removed. Soeder et al. (1978) showed that *Coelastrum proboscideum* absorbs 100% of lead from 1.0 ppm solution with 20 h at 23 °C and about 90% after only 1.5 h at 30 °C.

Cadmium was absorbed a little less efficiently, with about 60% of the cadmium being absorbed from a 40 ppb solution after 24 h. McHardy and George (1990) like Vymazal (1984), studied *Cladophora glomerata* in artificial freshwater channels and found that, the algae were excellent accumulators of zinc. There have also been reports of accumulation of Cu^{2+}, Pb^{2+} and Cr^{3+} as well as Ni^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+} and Mn^{2+} by algae (Chen et al., 2008; Gupta and Rastogi, 2008; Sari and Tuzen, 2008; Pahlavanzadeh et al., 2010; Gupta et al., 2010; Chakraborty et al., 2011; Lourie and Gjengedal, 2011; Kumar et al., 2012; Tastan et al., 2012; Piotrowska-Niczyporuk et al., 2012).

Algae in experimental rice paddles were found to accumulate and concentrate Cd^{2+} by a factor of about 1000 times when compared to the ambient (Reiniger, 1977; Liu et al., 2009). Algae are also good accumulators of compounds such as organochlorides and tributyl tin (Payer and Runkel, 1978; Wright and Weber, 1991). They have also been reported to break down some of these compounds (Lee, 1989; Wu and Kosaric, 1991).

Baeza-Squiban et al. (1990) and Schimdt (1991) have shown that the green alga *Dunaliella bioculata* produced an extracellular esterase which degrades the pyrethroid insecticide Deltamethrin. Algae have also been shown to degrade a range of hydrocarbons such as those found in oily wastes (Cerniglia et al., 1980; Carpenter et al., 1989).

11. Algae as a monitor of water quality

During the last three decades several investigations have described the algal bioassays in response to environmental perturbations and their use as indicative organisms of water quality (Mohamed, 1994). In 1959, Palmer published a composite rating of organisms such as *Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia* and *Navicula*, which could be used as indicators of water pollution, whereas the presense of different organisms such as *Lemanea, Stigeoclonium* and certain species of *Micrasterias, Staurastrum, Pinnularia, Meridion* and *Surirella* would indicate that the water sample would be considered unpolluted.

12. Alternative culture and treatment systems

12.1. Hyperconcentrated cultures

Hyperconcentrated cultures are cultures with an algal biomass $> 1.5\ g\ l^{-1}$. On a small-scale, experiments with hyperconcentrated cultures have shown that these can accelerate the removal of nutrients compared to normal cultures. Algae for such experiments are concentrated by flocculation and settling using a flocculent such as chitosan (Lavoie and De la Noüe, 1983; Morales et al., 1985). Cell concentrations of up to 1.9 g dry weight l^{-1} have been obtained for *Oscillatoria* sp. grown on sewage sludge (Hashimoto and Furukawa, 1989).

Working with *Scenedesmus obliquus* cultures have shown that great nitrogen removal was greatly accelerated for 1.9 g dry weight l^{-1} cultures compared to normal density cultures of 0.5 g dry weight l^{-1} (Lavoie and De la Noüe, 1985). They have also demonstrated that the rate of removal of ammonium and phosphorous in these hyperconcentrated cultures was proportional to algal concentration and independent of the obvious light limitation due to self-shading. Although this work has been carried out only on a small scale so far, the use of such hyperconcentrated cultures would require smaller pond areas, or would permit a reduced residence time, both of which have potential advantages. The engineering and economic feasibility of such systems on a large-scale remains to be determined.

12.2. Immobilized cell system

One of the major problems in the utilization of microalgae for the biological tertiary treatment of wastewater is their recovery from the treated effluent (Chevalier and De la Noüe, 1985a,b). Among the ways of solving this problem which have been recently studied are immobilization techniques (De la Noüe and Proulx, 1988). Immobilization appears to offer several advantages in comparison with batch or continuous fermentation where free microorganisms are used (Hall and Rao, 1989). Chevalier and De la Noüe (1985a,b) found that k-carrageenan-immobilized *Scenedesmus* cells were able to take up nitrogen and phosphorus at rates similar to those of free microalgae. Immobilized living cells possess some advantages in comparison with suspended cells; for example, immobilized microalgae on a suitable support simplify the treatment of liquid substances because of the entrapment of living cells, which contributes to increasing the cells retention time in the reactor (Travieso et al., 1992). It will be interesting to confirm the feasibility of using immobilized microalgae and cyanobacteria for removing nitrate, ammonium, and phosphate from high volume effluent discharges. It has been reported that *Phormidium laminosum* immobilized on polymer foam has the potential to remove nitrate in a continuous-flow system with uptake efficiencies above 90% (De la Noüe et al., 1990; Garbisu et al., 1991; Travieso et al., 1992; Sawayama et al., 1998). Sawayama et al. (1998) have reported that hollow fiber-immobilized cyanobacterial systems are easy to construct and immobilization does not take a long time. Also Markov et al. (1995) have reported that high rates of hydrogen production are made possible by using immobilized cyanobacteria on hollow-fiber immobilization systems could improve the removal efficiency of inorganic nutrients from treated wastewater.

Direct generation of electricity has also been demonstrated by immobilizing the cyanobacterial species *Mastigocladus* (Ochiai et al., 1980) and *Phormidium* (Ochiai et al., 1983) on SnO_2 optically transparent electrodes.

12.3. Dialysis cultures

In dialysis culture the algae are separated from the nutrient-containing medium by a semi-permeable dialysis barrier. Low molecular weight compounds diffuse across this barrier in response to a concentration gradient (Jensen, 1976; Marsot et al., 1991). High cell density cultures can be maintained for prolonged periods in system with a high membrane surface area/culture volume ratio, and the algal cells show very efficient rates of nutrient utilization (Ney et al., 1981 and Marsot et al., 1991). One advantage of dialysis culture is that they can serve to exclude inhibitory substances and it also allows the microbiologically pure culture of the algae. The latter is particularly important for the production of high quality large human consumption. Such a system has, as yet, not been applied to the use of wastewaters for algal culture, however, this type of system deserves critical evaluation.

12.4. Tubular photobioreactors

One of the most promising areas in the development of new reactor types is the tubular photobioreactors (Fig. 2). Basically, these reactors are a closed system consisting of a clear tube within which the algae grow. The algae are circulated by means of a pump and the system also has a gas exchange unit where CO_2 can be added and photosynthetically produced O_2 is stripped from the medium. If necessary, a heat exchanger is also added to either cool (in tropical areas) or heat (in temperate areas) the culture.

The concept of tubular reactors is not new. Simple reactors were already tested by Davis et al. (1953), and many of the modern systems are derived from the work of Pirt et al. (1983), although similar system had been used in Czechoslovakia at Trebon earlier to grow *Chlorella*.

Two basic kinds of systems are presently used consisting either of (a) straight tubes arranged flat on the ground or in long vertical rows (Pirt et al., 1983; Pirt, 1986; Torzillo et al.,

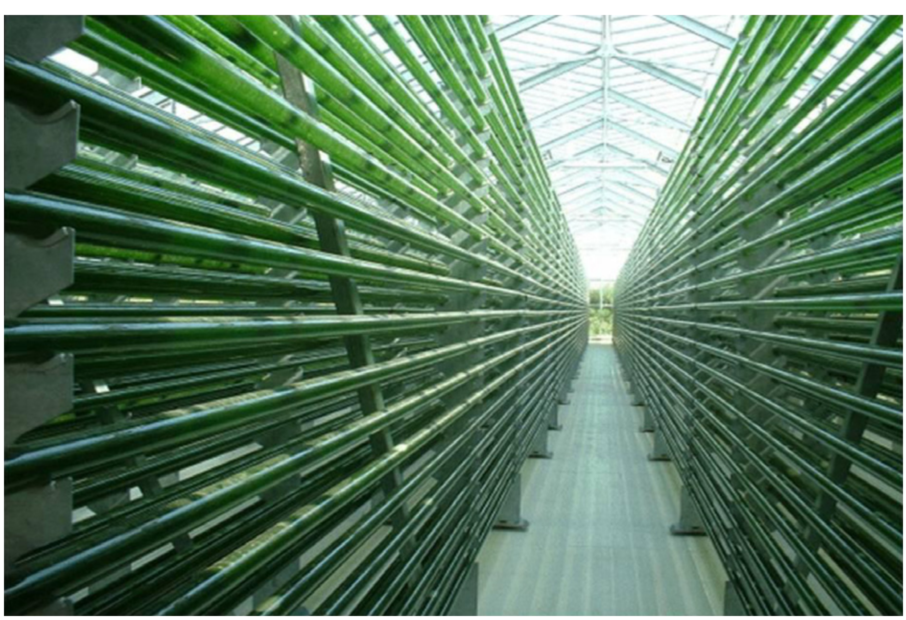

Figure 2 Schematic photobioreactor design, as follows a horizontal tubes.

1986; Bocci et al., 1988; Chaumont et al., 1988), or (b) of tubes spirally wound around a central support (Robinson et al., 1988; Borowitzka and Borowitzka, 1989b) or a similar helical structure (Lee and Bazin, 1990).

The tubes can be of glass, Perspex or PVC, and diameters range from about 24 cm to 24 mm. It is interesting to note that most systems are now tending to use the narrower diameter tubes, since these appear to have better hydrodynamic properties and result in improved productivity. Circulation of the algal culture is by means of diaphragm, peristaltic, lobe or centrifugal pumps or by an airlift. From an engineering point of view, the circular reactors are easier to construct, and occupy less land area per unit volume.

These photobioreactors have been used on a pilot scale to grow a wide variety of algae including *Spirulina*, *Porphyridium*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Tetraselmis* and *Phaeodactylum*. These reactors also have the advantage of almost linear scale-up, unlike paddle wheel and similar ponds, where scale-up presents major difficulties (Borowitzka and Borowitzka, 1989b). Tubular reactors have several potential problems which affect algal productivity. These are temperature control, control of O_2 and CO_2, growth of the algae on the inner surface of the tubes and adequate circulation speeds without damage to the relatively fragile algal cells.

12.5. Stabilization ponds

Waste Stabilization Ponds (WSP) have proven to be effective alternatives for treating wastewater, and the construction of low energy-consuming ecosystems that use natural processes, in contrast to complex high-maintenance treatment systems, will hopefully lead to more ecologically-sustainable wastewater treatment in future. CWs also have the capability of meeting the demand for a high percentage removal of pathogenic organisms, compared to conventional technologies. CWs combined, and joined with other technologies, may be important for even more improved performance of water cleaning systems. Many countries in tropical climates use WSPs for wastewater treatment (e.g., Tanzania, Kenya, Malawi, Uganda, Zambia, Botswana, Zimbabwe). Many of these systems have been performing below the required standards, due to lack of proper operation and maintenance (Kayombo et al., 1999).

Waste Stabilization Ponds (WSPs) are large, shallow basins in which raw sewage is treated entirely by natural processes involving both algae and bacteria. They are used for sewage treatment in temperate and tropical climates, and represent one of the most cost-effective, reliable and easily-operated methods for treating domestic and industrial wastewater. Waste stabilization ponds are very effective in the removal of faecal coliform bacteria. Sunlight energy is the only requirement for its operation. Further, it requires minimum supervision for daily operation, by simply cleaning the outlets and inlet works. The temperature and duration of sunlight in tropical countries offer an excellent opportunity for high efficiency and satisfactory performance for this type of water-cleaning system. Further, the advantage of these systems, in terms of removal of pathogens, is one of the most important reasons for its use.

12.5.1. Types of waste stabilization ponds

WSP systems comprise a single string of anaerobic, facultative and maturation ponds in series, or several such series in parallel. In essence, anaerobic and facultative ponds are designed for the removal of Biochemical Oxygen Demand (BOD), and maturation ponds for pathogen removal, although some BOD removal also occurs in maturation ponds and some pathogen removal in anaerobic and facultative ponds (Mara, 1987). In most cases, only anaerobic and facultative ponds will be needed for BOD removal when the effluent is to be used for restricted crop irrigation and fish pond fertilization, as well as when weak sewage is to be treated prior to its discharge to surface waters. Maturation ponds are only required when the effluent is to be used for unrestricted irrigation, thereby having to comply with the WHO guideline of >1000 faecal coliform bacteria/100 ml. The WSP does not require mechanical mixing, needing only sunlight to supply most of its oxygenation. Its performance may be measured in terms of its removal of BOD and faecal coliform bacteria.

12.5.1.1. Anaerobic ponds. Anaerobic ponds are (Fig. 3) commonly 2–5 m deep and receive wastewater with high organic loads (i.e., usually greater than 100 g BOD/m^3 day, equivalent to more than 3000 kg/ha day for a depth of 3 m). They normally do not contain dissolved oxygen or algae. In anaerobic ponds, BOD removal is achieved by sedimentation of solids, and subsequent anaerobic digestion in the resulting sludge. The process of anaerobic digestion is more intense at temperatures above 15 °C. The anaerobic bacteria are usually sensitive to pH < 6.2. Thus, acidic wastewater must be neutralized prior to its treatment in anaerobic ponds. A properly-designed anaerobic pond will achieve about a 40% removal of BOD at 10 °C, and more than 60% at 20 °C. A shorter retention time of 1.0–1.5 days is commonly used.

Figure 3 Anaerobic pond lined with a plastic membrane.

Figure 4 Facultative pond.

12.5.1.2. Facultative ponds. Facultative ponds (Fig. 4) (1–2 m deep) are of two types: Primary facultative ponds that receive raw wastewater, and secondary facultative ponds that receive particle-free wastewater (usually from anaerobic ponds, septic tanks, primary facultative ponds, and shallow sewerage systems). The process of oxidation of organic matter by aerobic bacteria is usually dominant in primary facultative ponds or secondary facultative ponds.

The processes in anaerobic and secondary facultative ponds occur simultaneously in primary facultative ponds, as shown in Fig. 2.1. It is estimated that about 30% of the influent BOD leaves the primary facultative pond in the form of methane (Marais 1970). A high proportion of the BOD that does not leave the pond as methane ends up in algae. This process requires more time, more land area, and possibly 2–3 weeks water retention time, rather than 2–3 days in the anaerobic pond. In the secondary facultative pond (and the upper layers of primary facultative ponds), sewage BOD is converted into "Algal BOD," and has implications for effluent quality requirements. About 70–90% of the BOD of the final effluent from a series of well-designed WSPs is related to the algae they contain.

In secondary facultative ponds that receive particle-free sewage (anaerobic effluent), the remaining non-settleable BOD is oxidised by heterotrophic bacteria (*Pseudomonas, Flavobacterium, Archromobacter and Alcaligenes* spp). The oxygen required for oxidation of BOD is obtained from photosynthetic activity of the micro-algae that grow naturally and profusely in facultative ponds.

Facultative ponds are designed for BOD removal on the basis of a relatively low surface loading (100–400 kg BOD/ha - day), in order to allow for the development of a healthy algal population, since the oxygen for BOD removal by the pond bacteria is generated primarily via algal photosynthesis. The facultative pond relies on naturally-growing algae. The facultative ponds are usually dark-green in colour because of the algae they contain. Motile algae (*Chlamydomonas* and *Euglena*) tend to predominate the turbid water in facultative ponds, compared to none-motile algae (*Chlorella*).

The algal concentration in the pond depends on nutrient loading, temperature and sunlight, but is usually in the range of 500–2000 μg chlorophyll-*a*/liter (Mara, 1987). Because of the photosynthetic activities of pond algae, there is a diurnal variation in the dissolved oxygen concentration. The dissolved oxygen concentration in the water gradually rises after sunrise, in response to photosynthetic activity, to a maximum level in the mid-afternoon, after which it falls to a minimum during the night, when photosynthesis ceases and respiratory activities consume oxygen. At peak algal activity, carbonate and bicarbonate ions react to provide more carbon dioxide for the algae, leaving an excess of hydroxyl ions. As a result, the pH of the water can rise to above 9, which can kill faecal coliform. Good water mixing, which is usually facilitated by wind within the upper water layer, ensures a uniform distribution of BOD, dissolved oxygen, bacteria and algae, thereby leading to a better degree of waste stabilization.

12.5.1.3. Maturation ponds. The maturation ponds, usually 1–1.5 m deep, receive the effluent from the facultative ponds. Their primary function is to remove excreted pathogens. Although maturation ponds achieve only a small degree of BOD removal, their contribution to nutrient removal also can be significant. Maturation ponds usually show less vertical biological and physicochemical stratification, and are well-oxygenated throughout the day. The algal population in maturation ponds is much more diverse than that of the facultative ponds, with non-motile genera tending to be more common. The algal diversity generally increases from pond to pond along the series (Mara, 1989). Although faecal bacteria are partially removed in the facultative ponds, the size and numbers of the maturation ponds especially determine the numbers of faecal bacteria in the final effluent. There is some removal of solids-associated bacteria in anaerobic ponds, principally by sedimentation. The principal mechanisms for faecal bacterial removal in facultative and maturation ponds are now known to be:

(a) Time and temperature;
(b) High pH (>9); and
(c) High light intensity, combined with high dissolved oxygen concentration.

Time and temperature are the two principal parameters used in designing maturation ponds. Faecal bacterial die-off in ponds increases with both time and temperature (Feachem et al., 1983). High pH values (above 9) occur in ponds, due to rapid photosynthesis by pond algae, which consumes CO_2 faster than can be replaced by bacterial respiration. As a result, carbonate and bicarbonate ions dissociate, as follows:

$$2HCO)_3^- \rightarrow CO_3^{2-} + H_2O + CO_2$$
$$CO_3^{2-} + H_2O_2 \rightarrow 2OH^- + CO_2$$

The resulting CO_2 is fixed by the algae, and the hydroxyl ions accumulate, often raising the pH to values above 10. Faecal bacteria (with the notable exception of Vibrio cholerae) die very quickly at pH values higher than 9 (Pearson et al., 1987a,b). The role of high light intensity and high dissolved oxygen concentration has recently been elucidated (Curtis et al., 1992). Light of wavelengths between 425 and 700 nm can damage faecal bacteria by being absorbed by the humic substances ubiquitous in wastewater. They remain in an excited state sufficiently long to damage the cell. Light-mediated die-off is completely dependent on the presence of oxygen, as

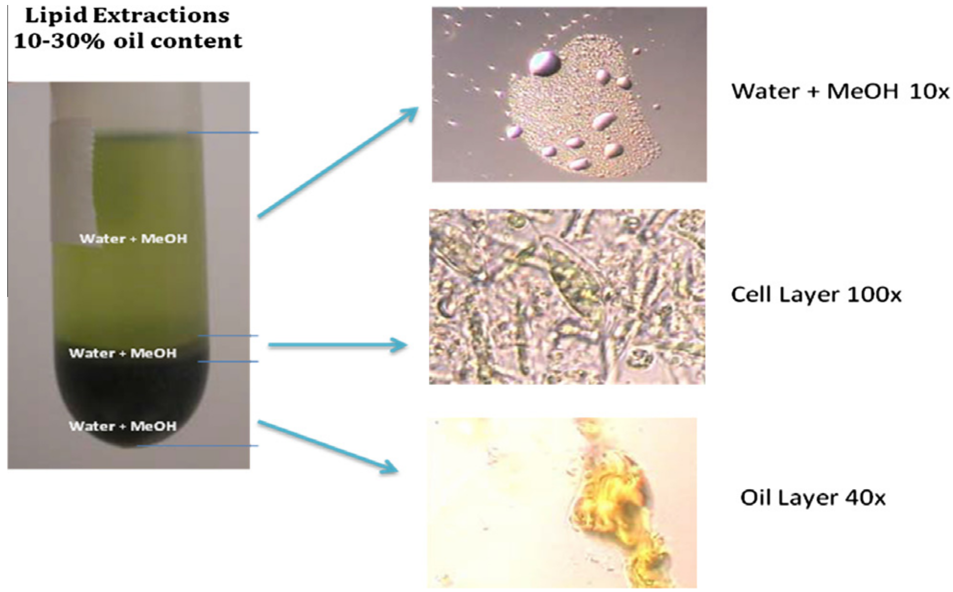

Figure 5 Biofuel production by microalgae.

well as being enhanced at high pH values. Thus, the sun plays a threefold role in directly promoting faecal bacterial removal in WSP, and in increasing the pond temperature, and more indirectly by providing the energy for rapid algal photosynthesis. This not only raises the pond pH value above 9, but also results in high dissolved oxygen concentrations, which are necessary for its third role; namely, promoting photo-oxidative damage.

12.6. Algal mats

All the systems considered so far have used microalgae. An alternative system for nutrient removal from wastewaters is to use attached macroalgae or other aquatic plants. One such system is the algal mat system developed by Adey (1982), and which is being used to remove nutrients from large tropical aquarium systems such as those at Reef World and at the James Cook University in Townsville. In this system, the algae (a range of turf-forming species such as *Enteromorpha*, *Cladophora*, *Sphacelaria*, *Ectocarpus*, *Ceramium*, *Polysiphonia*, *Herposiphonia* and *Oscillatoria*) are grown on a net or mesh and the nutrient-rich water is passed over them. The algae containing the nutrients are regularly removed by mechanically removing them from the mats. Although this system has proven very effective in controlling the nutrient levels in the aquarium water so that even corals, which are very sensitive to elevated nutrient levels, can grow, it does require a large surface area and is very labor intensive. In certain months of the year the natural daylight also has to be supplemented with artificial lighting to maintain an adequate rate of nutrient removal.

Other aquatic plant based systems have been also proposed for nutrient removal using aquatic plants such as water hyacinth, *Typha* and *Phragmites*, however all of these systems have been shown to be less efficient than algal systems (Werblan et al., 1978; Wolverton, 1982; Finlayson and Chick, 1983 and Finlayson et al., 1987).

13. Utilization of harvested algae biomass in biogas production

Waste-grown microalgae are a potentially important biomass for biofuel production. However, most of the wastewater treatment ponds systems do not use algae harvesting. Those that do, typically return the biomass to the ponds, where it decomposes on the pond floor, releasing methane to the atmosphere and degrading water quality (Chaiprasert, 2011). Instead, the algae biomass could be processed for lipid extraction to be used in transportation fuel, or it can be anaerobically digested to make biogas (US, DOE, 2009; Brune et al., 2009) (Fig. 5).

Waste-grown algae have widely varying lipid contents, and the technologies for lipid extraction are still under development (Woertz et al., 2009). Thus, anaerobic digestion is likely to be the near-term, appropriate use of algae biomass at wastewater treatment plants. However, algae typically yield less methane than wastewater sludge ($\sim$0.3 vs. 0.4 L CH_4/g volatile solids introduced). Ammonia toxicity and recalcitrant cell walls are commonly cited causes of the lower yields. Ammonia toxicity might be counteracted by co-digesting algae with high-carbon organic wastes. Carbon-rich feedstocks that are available near major wastewater pond systems include primary and secondary municipal sludge, sorted municipal organic solid waste, waste fats–oils greases (FOGs), food industry waste, waste paper, and various agricultural residues. Acclimation of the digester microbial community to algae digestion may also improve the yield.

Microalgae have two major advantages over higher plants with respect to biofuels production. First, biomass productivities are significantly greater for microalgae, with productivities projected at about 70 metric tons per hectare-year of ash-free dry weight (i.e. organic matter) in specialized growth reactors, such as high rate ponds (Sheehan et al. 1998). This productivity compares well with terrestrial temperate crops (e.g., 3 MT/ha yr for soybeans, 9 MT/ha yr for corn, and 10–13 MT/ha yr for switchgrass or hybrid poplars (Perlack et al., 2005). Second, the cultivation of microalgae does not require arable land or fresh water – it can be carried out in shallow ponds on hardpan soils, using saline or brackish water. Relatively few studies have been published on the anaerobic digestion of microalgae (reviewed recently by Sialve et al., 2009). The earliest work compared digestion of domestic wastewater sludge and green microalgal biomass, *Scenedesmus* and *Chlorella*, harvested from wastewater ponds (Golueke et al., 1957). They found that these algae could yield as much as 0.25–0.50 L CH_4/g VS input at an 11-day retention time when incubated at 35–50 °C. (Methane yield is typically expressed as liters of methane produced per gram of volatile solids introduced into a digester.) The lower value was 32% less than the yield from the wastewater sludge. In addition, the maximum VS destruction was about 45% for the algae, compared to 60% for the wastewater sludge. They suggested that the relatively low digestability and thus yield of microalgal biomass was the result of cell walls resisting bacterial degradation, but being more readily digested by bacteria at the higher temperature. Methane yield and productivity were doubled when equal masses of wastewater sludge and *Spirulina* biomass were co-digested (Samson and LeDuy 1983). Similarly, Yen (2004) and Yen and Brune (2007) added waste paper (50% w/w) to aquacultural microalgal sludge to adjust the C:N ratio to around 20–25:1 which, in turn, doubled the methane production rate from 0.6 L/L day to 1.2 L/L day at 35 °C and with a hydraulic retention time of 10 days.

14. Conclusion

* Algae can be used in wastewater treatment for a range of purposes, including;
  1. reduction of BOD,
  2. removal of N and/or P,
  3. inhibition of coliforms,
  4. removal of heavy metals

* The high concentration of N and P in most wastewaters also means these wastewaters may possibly be used as cheap nutrient sources for algal biomass production. This algal biomass could be used for:
  1. methane production,
  2. composting,
  3. production of liquid fuels ((pseudo-vegetable fuels),
  4. as animal feed or in aquaculture and
  5. production of fine chemicals.

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